

Caspase immunofluorescence staining protocol

Apoptosis is a highly regulated mechanism of cell death, which converges on caspase activation. Immunofluorescence is a useful technique when you want to detect caspases and other apoptosis-related proteins simultaneously in a cell sample.

Materials required:

- Primary antibody against caspase e.g. [Active Caspase 3 antibody, rabbit pAb \(ab2302\)](#)
- Prepared, fixed samples on slides
- Triton X-100
- PBS
- Blocking buffer (PBS/0.1% Tween 20 + 5% appropriate* serum)
- Conjugated secondary antibody e.g. [goat anti-rabbit Alexa Fluor® 488 conjugate \(ab150077\)](#)
- Mounting medium
- Humidified chamber

*We recommend using serum from the host species of the secondary conjugate antibody (or closely related species) e.g. if using a goat anti-rabbit conjugate, use goat serum in the blocking buffer.

Procedure:

- 1 Permeabilize the fixed samples by incubating in PBS/0.1% Triton X-100 for 5 min at room temperature.
- 2 Wash three times in PBS, for 5 min at room temperature.
- 3 Drain the slide and add 200 µL of blocking buffer (PBS/0.1% Tween 20 + 5% appropriate* serum). Lay the slides flat in a humidified chamber and incubate for 1-2 hr at room temperature. Rinse once in PBS.
- 4 Add 100 µL of the primary antibody diluted 1:200 in blocking buffer. You can also prepare a slide with no primary antibody as a negative control. Incubate slides in a humidified chamber overnight at 4°C.
- 5 The following day, wash the slides three times, 10 min each in PBS/0.1% Tween 20 at room temperature.
- 6 Drain slides and add 100 µL of appropriate secondary conjugated antibody diluted 1:500 in PBS. Lay the slides flat in a humidified chamber, protected from light, and incubate for 1-2 hr at room temperature. Wash three times in PBS/0.1% Tween 20 for 5 min, protected from light.
- 7 Drain the liquid, mount the slides in a permanent or aqueous mounting medium and observe with a fluorescence microscope.

This protocol should be used as a guide. Optimization will be required depending on the sample and antibodies used. Our antibody datasheets provide suggested working concentrations, which should be tested in your own experiments.

For a simple and convenient way of measuring caspase activity, find our range of caspase activity kits at www.abcam.com/kits/apoptosis-assay-kits

An optimized protocol is provided with each kit, and should be followed carefully.